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Letters

Allosteric Inhibitors of Hepatitis C Polymerase: Discovery of Potent and Orally Bioavailable Carbon-Linked Dihydropyrones

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Abstract: The discovery and optimization of a novel class of carbon-linked dihydropyrones as allosteric HCV NS5B polymerase inhibitors are presented. Replacement of the sulfur linker atom with carbon reduced compound acidity and greatly increased cell permeation. Further structure−activity relationship (SAR) studies led to the identification of compounds, exemplified by **23** and **24**, with significantly improved antiviral activities in the cell-based replicon assay and favorable pharmacokinetic profiles.

Hepatitis C virus (HCV) is a major public health problem that infects an estimated 170 million people worldwide.[1] It is the leading cause of liver transplantation in the developed world and results in over 10 000 deaths annually in the U.S. alone. Currently there is no vaccine to prevent the disease nor specific antiviral agent directed against HCV infection on the market. The standard treatment of chronic hepatitis C is based on the combination of subcutaneous pegylated interferon (Peg-IFN) along with the oral nucleoside drug ribavirin. However, serious side effects and poor response rates, particularly among patients with genotype 1, render the development of novel anti-HCV therapy an urgent need.[2]

In the past decade, particularly after a reliable tissue culture replication system based on subgenomic replicons was introduced,[3] there have been tremendous efforts dedicated to the development of novel, effective anti-HCV agents.[4,5] Among essential enzymes for HCV replication, the HCV NS5B polymerase,[6] along with HCV NS3/4 protease,[7] has attracted the most attention as a potential therapeutic target. Both nucleoside and non-nucleoside HCV NS5B polymerase inhibitors, alone or in combination with Peg-IFN, have demonstrated efficacy in human clinical trials (Figure 1). A 15-day monotherapy trial in genotype 1 patients with the Idenix nucleoside inhibitor valopicitabine **1** (NM-283) resulted in a mean viral load reduction of 1.2 logs with no apparent resistance development. When combined with Peg-IFN, valopicitabine achieved a 4.5 log reduction in viral load at 24 weeks.[8] Recently, interim results from an early clinical trial of Roche's nucleoside inhibitor **2** (R1626) also indicated human efficacy.[9] The efforts in developing non-nucleoside polymerase inhibitors generated equally encouraging results. Compound **3** (HCV-796), an allosteric-site inhibitor from Viropharma/Wyeth, was shown to induce a mean viral load reduction of 1.4 logs in monotherapy and 2−3 logs in combination therapy with Peg-IFN after 14 days in patients infected with various genotypes.[10] These results constitute proof of concept for HCV polymerase inhibitors and support the possibility of replacing ribavirin from the current standard therapy with a more tolerable alternative.

In the previous communication,[11] we have reported the identification and preliminary optimization of a novel class of dihydropyrones derived from a high-throughput screening (HTS) hit (Figure 2, **4**) as potent HCV NS5B polymerase inhibitors. These compounds bind at an allosteric binding site close to the junction of thumb and finger domain, approximately 30 Å away from the active site.[12] In particular, a more optimized sulfur-linked analogue, 6-[2-(3-chloro-4-methoxyphenyl)ethyl]-6-cyclopentyl-3-[(5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-thio]-4-hydroxy-5,6-dihydro-2*H*-pyran-2-one (Figure 2, **5**), exhibits low nanomolar potency in the biochemical assay against the isolated HCV NS5B polymerase and is also effective at blocking the replication of subgenomic HCV RNA in the replicon system. However, significant hurdles still remain for the development of such compounds as drug candidates. While single-digit nanomolar potency was achieved in the enzymatic assay against the truncated genotype 1b HCV NS5B Δ21 polymerase, the S-linked dihydropyrones only demonstrated moderate antiviral activity in the cell-based replicon assay at micromolar concentrations.[13] Additionally, in the subsequent rat pharmacokinetic evaluation, **5** showed low bioavailability (2%, Table 1) and poor

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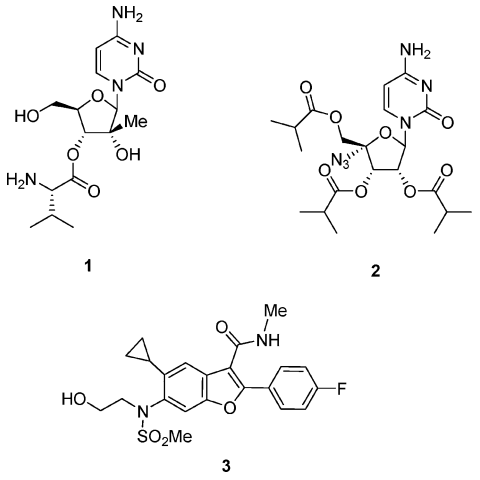


Figure 1. Selected HCV NS5B polymerase inhibitors that demonstrated efficacy in human clinical trials.

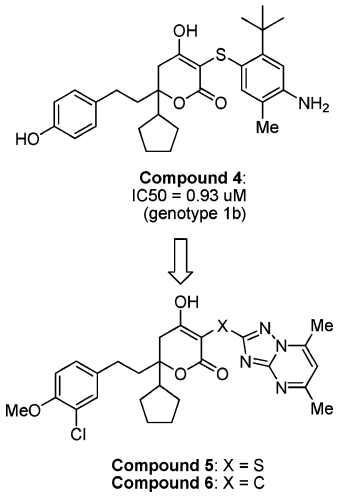


Figure 2. Dihydropyrones as HCV polymerase inhibitors.

Table 1. Comparison between S-Linked (**5**) and C-Linked (**6**) Dihydropyrones

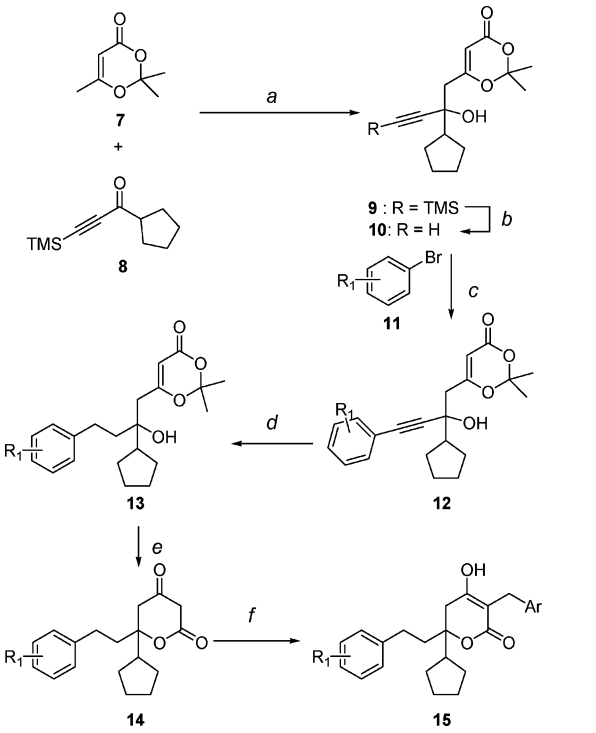
	X = S (**5**)	X = C (**6**)
IC_{50} (μM)[a]	0.036	0.020
EC_{50} (μM)[a]	3.25	0.33
EC_{50}/IC_{50}	90	17
CC_{50} (μM)	>100	>100
po dose (mg kg^{-1})[b]	25	10
iv dose (mg kg^{-1})[b]	5	2
Cl (mL min^{-1} kg^{-1})[c]	14.8 ± 2.6	8.3 ± 1.1
V_{dss} (L kg^{-1})[d]	2.0 ± 0.5	0.3 ± 0.05
$T_{1/2}$ effective (h)	1.5 ± 0.2	0.5 ± 0.27
F_a (%)[e]	2.5	48
F (%)[f]	2	42
Caco-2 AB/BA Papp × 10^{-6} (cm s^{-1})	3/2.3	10/6.8

[a] Genotype 1b. [b] 70% polyethylene glycol with 30% saline as dosing vehicle. [c] Total plasma clearance. [d] Volume of distribution. [e] Fraction absorbed. [f] Bioavailability.

absorption after oral dosing (2.5% fraction absorbed). In this report, we describe the discovery and structure−activity relationships (SAR) of a series of carbon-linked dihydropyrones that achieved good potency in the biochemical assay and cell-based replicon system and also demonstrated favorable profiles in rat pharmacokinetic studies.

The observation that S-linked dihydropyrones generally exhibit large gaps between their potency in the cell-based replicon system and enzymatic assays (as measured by the ratio

Scheme 1. Synthesis of Carbon-Linked Dihydropyrone[a]



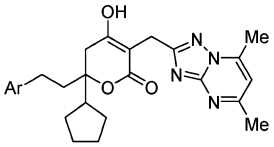
[a] Reagents and conditions: (a) LDA, −78 °C; (b) CsF, MeOH, 25 °C, 61% for two steps; (c) $Pd(PPh_3)_2Cl_2$, CuI, diisopropylamine, DMF, 90 °C; (d) $Pd(OH)_2$, H_2, MeOH, 25 °C; (e) K_2CO_3, MeOH, 45 °C; (f) ArCHO, MeOH, BH_3·SMe_2, 25 °C.

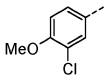
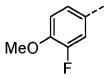
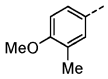
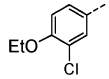
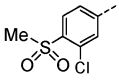
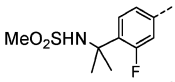
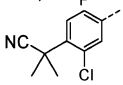
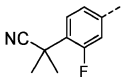
of EC_{50} to IC_{50}) and the fact that compounds showed low absorption in rat PK studies turned our attention to the permeability of this series of compounds. In experimental pK_a determinations, the S-linked dihydropyrones (p$K_a \approx 4-5$) are typically 10- to 100- fold more acidic than the corresponding C-linked analogs (p$K_a \approx 5-7$). To evaluate the impact of the single linker atom replacement on the overall profile of these molecules, the carbon-linked dihydropyrone **6** was prepared through a convergent synthetic sequence as outlined in Scheme 1.

2,2,6-Trimethyl-4*H*-1,3-dioxin-4-one (**7**) was deprotonated with LDA at −78 °C in THF, which upon treatment with TMS-protected ketone **8** provided acetylene **9**. After removal of the TMS group under CsF conditions in MeOH, the resulting alkyne **10** served as a common intermediate for all further analogue synthesis. Installation of the aromatic group at the C-terminal of alkyne **10** was accomplished by a Pd-catalyzed Sonogashira coupling reaction with a variety of different bromides **11**. Hydrogenation of the triple bond in **12** with $Pd(OH)_2$ in MeOH generated the saturated **13**, which subsequently reacted with K_2CO_3 at 45 °C to afford the desired dihydropyrone scaffold through a ring closure reaction in good overall yield. To generate the carbon-linked dihydropyrones, we developed a novel, efficient coupling reaction between the unsubstituted dihydropyrone **14** and aromatic aldehydes in the presence of borane−dimethyl sulfide complex in MeOH at ambient temperature, presumably through a condensation/reduction sequence. This one-step protocol provided the desired carbon-linked analogues **15** in consistently high yields with operational simplicity and offered a significant advantage over the previously reported procedure.[14]

The compounds synthesized were assayed against the HCV genotype 1b NS5B polymerase to assess their inhibitory activity (IC_{50}) as previously described.[11] The in vitro antiviral activity

Table 2. Further Optimization of C-Linked Dihydropyrones



Compd	Ar	IC_{50} (uM)[a]	EC_{50} (uM)[a]	CC_{50} (uM)[a]
6		0.020	0.33	>32
16		0.015	0.40	>32
17		0.054	1.2	>32
18		0.004	0.32	>32
19		0.060	0.61	>32
20		0.017	0.11	>32
21		0.002	0.074	>32
22		0.003	0.030	>32

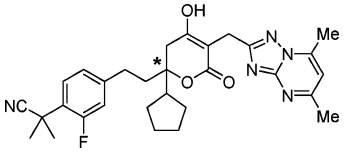
[a] Genotype 1b.

(EC_{50}) and cytotoxicity (CC_{50}) were determined in the Huh-7 hepatoma cell line harboring a self-replicating HCV subgenomic replicon of genotype 1b.[3]

As summarized in Table 1, replacement of the sulfur linker atom with carbon had pronounced effects on the overall compound profiles. While in the enzymatic assay, the C-linked **6** showed only a 2-fold advantage over the S-linked analogue **5** and the improvement in the cell-based replicon system was 10-fold, thus dramatically narrowing the ratio of the EC_{50} to IC_{50} (17 for **6** vs 90 for **5**). These findings were well in line with the permeability of these compounds measured in Caco-2 cell lines: switching from S-linker to C-linker significantly increased the cellular permeation from low/moderate to high (Caco-2 AB, 3×10^{-6} cm/s for **5** and 10×10^{-6} cm/s for **6**), and both compounds did not exhibit efflux issues. Encouraged by these results, **5** and **6** were subjected to rat in vivo pharmacokinetic evaluation. The C-linked **6** demonstrated a much better oral absorption, as measured by the fraction absorbed ($F_a = 48\%$) and good overall bioavailability at 42%.

In view of its improved potency in the replicon system and superior pharmacokinetic profile, the C-linked dihydropyrone **6** was judged to be a more appealing lead for further SAR studies. Optimization of the aromatic region at the left-hand side of the molecule is summarized in Table 2. While substitution of the chlorine atom at the meta position in **6** with fluorine (Table 2, **16**) was well tolerated, the corresponding methyl replacement resulted in a significant reduction in potency, particularly in the cell-based replicon assay (**17**, $EC_{50} = 1.2$ μM). A larger ethoxy group at the para position (**18**, $IC_{50} = 0.004$ μM) provided a 5-fold improvement over the methoxy analogue (**6**) in the enzymatic assay but did not offer an obvious advantage in the cell-based replicon assay. Replacement of the methoxy group in **6** with a methylsulfone provided **19**, which suffered a 3-fold loss in activity in the biochemical and replicon assays. Incorporation of a *gem*-dimethylmethanesulfonamide moiety at the para position of the phenyl ring resulted in **20**, which boosted the replicon activity by 4-fold over **16**. Further exploration in this region led to the discovery of a *gem*-dimethyl cyano group that significantly improved compound potency by making a direct hydrogen bond interaction with nearby Leu 497. Both **21** and **22** achieved single-digit nanomolar potency against the HCV polymerase enzyme, while the fluorine analogue (**22**) was about 2-fold more potent than the chlorine analogue (**21**) in the cell-based replicon assay.

Table 3. Profiles of Two Enantiomers of Compound **22**



	enantiomer 1 (**23**)	enantiomer 2 (**24**)
IC_{50} (μM)[a]	0.003	0.001
IC_{50} (μM)[b]	0.070	NA
EC_{50} (μM)[a]	0.015	0.032
CC_{50} (μM)[a]	320	320
po dose (mg kg^{-1})[c]	10	10
iv dose (mg kg^{-1})[c]	2	2
Cl (mL min^{-1} kg^{-1})[d]	3.7 ± 0.8	4.0 ± 1.1
V_{dss} (L kg^{-1})[e]	0.3 ± 0.8	0.17 ± 0.1
$T_{1/2}$ effective (h)	0.88 ± 0.1	0.47 ± 0.2
F (%)[f]	31	37

[a] Genotype 1b. [b] Genotype 1a. [c] 70% polyethylene glycol with 30% saline as dosing vehicle. [d] Total plasma clearance. [e] Volume of distribution. [f] Bioavailability.

Racemic **22** was separated into its individual enantiomers using chiral HPLC with a Chiralpak AS-RH column. The two enantiomers showed comparable potencies in the enzymatic and replicon assays. The pharmacokinetic properties of enantiomers **23** and **24** were evaluated in rats, and their results are summarized in Table 3. Both compounds demonstrated low in vivo clearance and favorable oral bioavailability and half-lives. The low volumes of distribution exhibited by both enantiomers were anticipated as a result of the acidic nature of the dihydropyrone template. On the basis of its potency and pharmacokinetic profile, **23** was selected to be scaled up to kilogram-scale for further evaluations.[15]

In conclusion, we have described the discovery and optimization of a novel series of carbon-linked dihydropyrones as potent allosteric inhibitors of the HCV NS5B polymerase. Replacement of the sulfur linker with a carbon atom reduced the acidity of the dihydropyrone scaffold and significantly improved cellular permeation. This has led to an over 100-fold improvement in antiviral activities in the cell-based replicon assay and favorable rat pharmacokinetic profiles.

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Supporting Information Available: Experimental procedures and characterization data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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